

A Study of Stomatal Structure in Pandanaceae

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ABSTRACT: Many species of Pandanaceae have unspecialized stomata. In species of *Pandanus* there is a range of stomatal structure involving increasing elaboration of papillae arising from subsidiary and neighbouring cells so that guard cells, which are otherwise constant in structure throughout the family, are increasingly provided with a canopy of papillae which, together with sinking of the stomatal apparatus, produces an outer stomatal chamber. In the most specialized stomata this chamber is itself virtually occluded by lobed papillae.

Freycinetia may show significant differences from *Pandanus*. This linear trend of specialization can often be partly or wholly demonstrated on a single individual because stomata are always unspecialized on the first scalelike leaves of each renewal shoot, thereafter being increasingly specialized on subsequent leaves until the maximum *potential* elaboration characteristic of the species is realised on foliage leaves. This is briefly discussed in relation to taxonomy, physiology, and ecology.

THIS ARTICLE is the partial result of a recent study of the systematic anatomy of the Pandanaceae in which limited material of all three of its genera, *Freycinetia*, *Pandanus*, and *Sararanga*, has been examined. Insufficient material has been studied to permit worthwhile comment about the diagnostic value within the family of microscopic structures, except that anatomy is relatively uniform throughout the Pandanaceae and emphasizes its naturalness. In the survey a considerable range in stomatal structure was found, especially in *Pandanus*, a range not fully described by previous observers like Köfler (1918), Solla (1884), or Solereder and Meyer (1933). Observations were extended to indicate the range of stomatal structure throughout a single plant, whereupon it was discovered that, in certain instances, the full range of stomatal variation within the genus could be demonstrated within a single individual. This can be explained when the growth habit of *Pandanus* is understood.

It was felt that a general summary of stomatal structure in Pandanaceae would be a significant contribution to the literature on monocotyledonous anatomy. The present account deals

largely with *Pandanus*, notes on *Freycinetia* and *Sararanga* being included when they show outstanding features. Material of some 50 species has been examined during the course of this study, which—in view of the richness of *Pandanus*, estimated at 500–600 species, and of *Freycinetia*, at 180–200 species (St. John, 1960)—indicates how provisional this study is. It is not, therefore, intended as an exhaustive survey of the subject, but gives a clear indication of the known range of stomatal structure in Pandanaceae and points the way to more intensive study.

MATERIAL AND METHODS

The survey is largely based on herbarium material of *Pandanus* supplied from specimens in the Bernice P. Bishop Museum, Honolulu, Hawaii, by Dr. B. C. Stone, the material being selected to represent a wide range of subgenera. It was supplemented by material collected by myself and others in the wild, and by material in cultivation at the Fairchild Tropical Garden and the U. S. Department of Agriculture Experiment Station, Old Cutler Road, Miami, Florida. Slides and specimens in the collections of the Jodrell Laboratory, Royal Botanic Gardens, Kew,

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Manuscript received September 27, 1963.

England, have also been examined. The cultivated material was in many instances unnamed and was used largely to confirm observations on named species and to provide large samples for studies in stomatal variability. Observation on unnamed species, with few exceptions, has not been tabulated in this account. A full list of named material and sources is given in the appendix.

Only simple technical methods have been used. Dried material was restored to a consistency suitable for anatomical study by boiling for several minutes in water to which a little Clorox was added. This revived material was stored in formalin-acetic-alcohol and proved quite satisfactory for anatomical study. Some distortion which could not be eliminated by the reviving technique did not obscure anatomical details. This point is emphasized, because it means that stomatal studies in Pandanaceae can proceed satisfactorily using herbarium material. Fresh material has been examined either unfixed or fixed in formalin-acetic-alcohol.

Preparations showing the surface layers in surface view were made by the simple scraping technique employed at the Jodrell Laboratory and described in detail by Metcalfe (1960:lx). Such preparations were either made permanent in Canada Balsam, after staining in safranin and Delafield's haematoxylin, or were examined as temporary mounts in chlor-zinc-iodide. All material to be sectioned was desilicified by soaking overnight in 50% hydrofluoric acid, followed by prolonged washing in running water. Freehand sections of the demineralized but unembedded material, in 70% alcohol, were cut at 10–20 μ in transverse and longitudinal sections on a Reichert sliding microtome. Most observations were made on sections in temporary glycerine mounts, either unstained or after treatment with a variety of temporary stains, such as phloroglucin and concentrated hydrochloric acid, Sudan IV, chlor-zinc-iodide, iodine-potassium iodide, and 70% sulfuric acid. A few permanent preparations of the freehand sections were made by a method described elsewhere (Tomlinson, 1961: 5).

Illustrations (Figs. 8–51) are from camera lucida drawings. These are somewhat stylized because of some persistent distortion in dried

material and because it was often possible to cut only thick sections. This is quite an advantage since they allowed a complete three-dimensional picture of stomata to be built up from optical sections in three planes at right angles.

GENERAL MORPHOLOGY OF PANDANACEAE

This has been described by Schumann (1897), Warburg (1900), and Schoute (1903, 1906) and summarized briefly by Tomlinson (1964). Some knowledge of growth habit and branching in Pandanaceae is essential for an understanding of variation in stomatal structure throughout a single individual. Early branching begins independently of flowering, but later branching is regular, sympodial, and largely dependent upon flowering. Lateral branches then arise below and replace a parent axis, growth of which is ended by its conversion into a terminal inflorescence. Eviction of terminal buds by one or more laterals is a method of branching typical of plants without secondary tissue (Schoute, 1906; Tomlinson, 1964). In Pandanaceae each lateral renewal branch in these sympodia bears a similar sequence of leaf forms (Fig. 1). The first leaf on a branch is always a short, bicarinate prophyll in the adaxial (adossierte) position. This is succeeded by a number of scale leaves, each successively longer than its predecessor, forming a gradual transition to the normal foliage leaves. Length of foliage leaves is fairly constant, although distal foliage leaves, close to the inflorescence, are short. Renewal buds, one or more of which grow out to form the next segments of the sympodium, are prominent in the axils of these distal leaves. Change of the vegetative into a reproductive axis is marked by elongation and narrowing of internodes. Inflorescence leaves, which are short foliage leaves with a narrow insertion on the lowest nodes of the inflorescence, may be distinguished (Schumann, 1897). Beyond them is a rapid transition to short, colorless bracts. The over-all leaf sequence illustrated diagrammatically in Figure 1 for one sympodial segment of a small *Pandanus* is characteristic for all Pandanaceae, although the number of leaves on each segment varies considerably, even within a single individual.

Foliage leaves are lanceolate. In *Pandanus* and

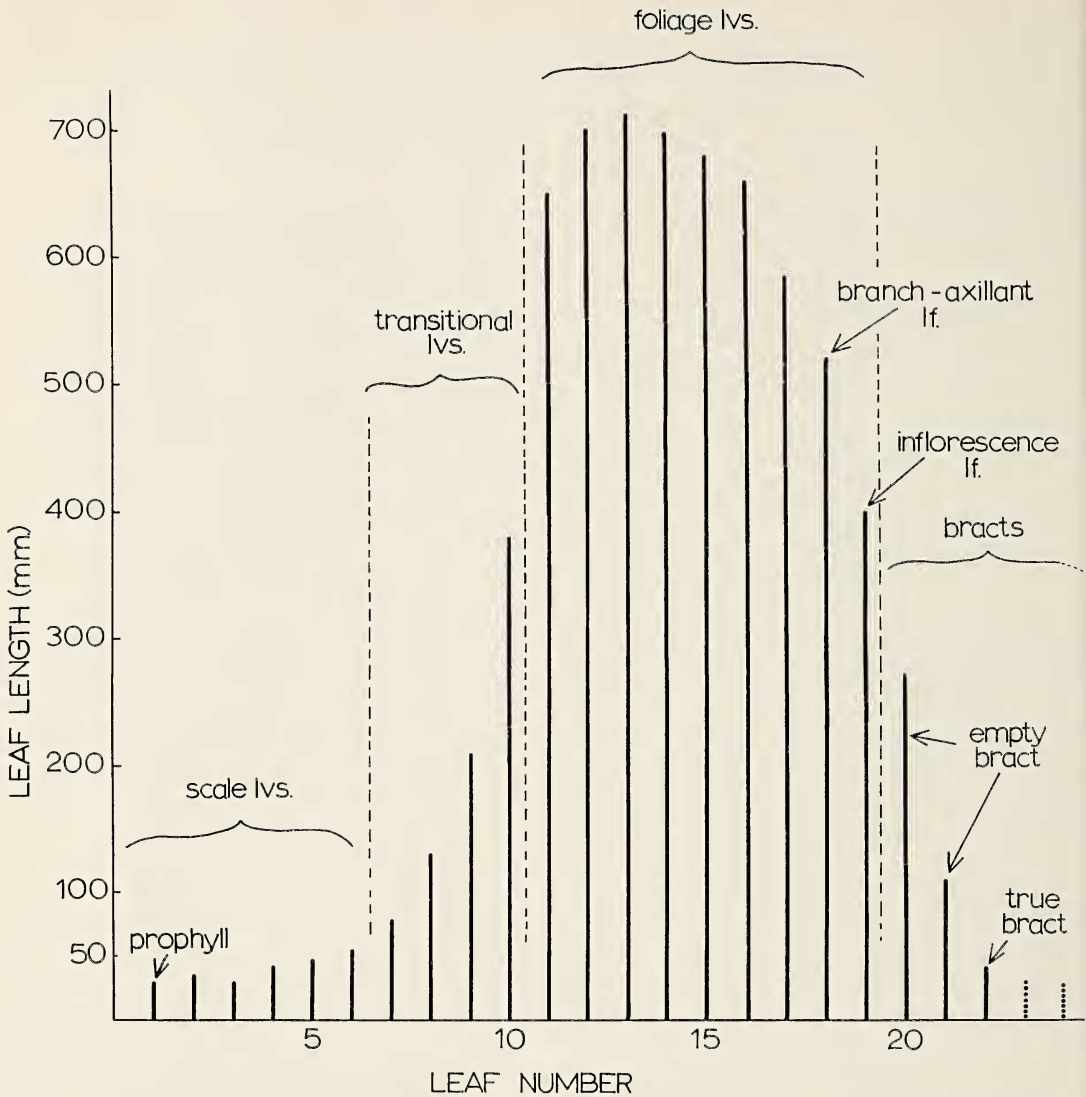


FIG. 1. Diagrammatic representation of change in leaf length along a single shoot in a diminutive species of *Pandanus* (P. I. 135008 U.S.D.A. Experiment Station, Miami, Florida).

Sararanga they more or less completely enclose the axis at their insertion without forming a closed tube; there is no distinction between blade and sheath. In *Freycinetia* there is a slight differentiation between a short basal sheathing region and a blade, the mouth of the open sheath being slightly auriculate.

GENERAL ANATOMY OF THE LEAF

Leaf anatomy is very uniform throughout the Pandanaceae so that all leaves are basically constructed alike, differences between different spe-

cies being largely quantitative.

The leaf is dorsiventral. Hairs are absent, although leaf margins and midrib usually bear prominent spines. Stomata are largely confined to the abaxial surface. The leaf is delimited by well-developed surface layers, each consisting of a shallow, strongly cutinized but never markedly thick-walled epidermis and well-developed, colourless, and often slightly thick-walled hypodermal layers, usually three or four cells deep. Adaxial hypodermal layers are usually somewhat thicker than abaxial. Epidermal cells are usually

rectangular, slightly longitudinally extended, and arranged in longitudinal files. In the abaxial, but not adaxial, epidermis distinct nonstomate costal regions below the veins are differentiated from intercostal regions, between the veins, to which stomata are restricted. There is no coincidence between epidermal and hypodermal files. Hypodermal cells contrast with epidermal in that their outermost two layers consist of flattened cells extended transversely and not longitudinally to the long axis of the leaf. Since epidermal cells are thus much narrower than hypodermal cells in the transverse direction, three or four files of epidermal cells occupy the space of one file of outer hypodermal cells (Fig. 2). Anticlinal walls in outer hypodermal layers do not coincide since their cells are clearly derived from different initial layers. The outermost hypodermal layers often become sclerotic; the inner cells in contrast are larger, more cubical in shape, and usually remain thin-walled. Hypodermal layers are continuous except for well-developed substomatal chambers by which the stomata communicate with the internal leaf atmosphere.

The mesophyll includes equivalent, parallel, longitudinal veins, separated by assimilating layers which surround pseudolacunae. Each vein is supported by a well-developed parenchymatous and fibrous buttress, continuous with the hypodermis of each surface. Vascular tissues are quite complex but are not considered further in this account. The mesophyll between the veins is represented by large colourless cells which tend to be arranged in separate transverse plates. These cells collapse somewhat in mature leaves, so that in thin transverse sections there is an apparent lacuna between each adjacent pair of veins. These pseudolacunae are lined by small chlorenchyma cells, the adaxial layers often conspicuously palisade-like. The lacunae are also traversed by compact transverse diaphragms, mostly two cells thick. At frequent intervals these diaphragms include transverse vascular commissures which anastomose with the longitudinal veins. Strands of fibres, independent of the fibrous extensions of the bundle sheath, occur in a number of species, usually in the superficial layers.

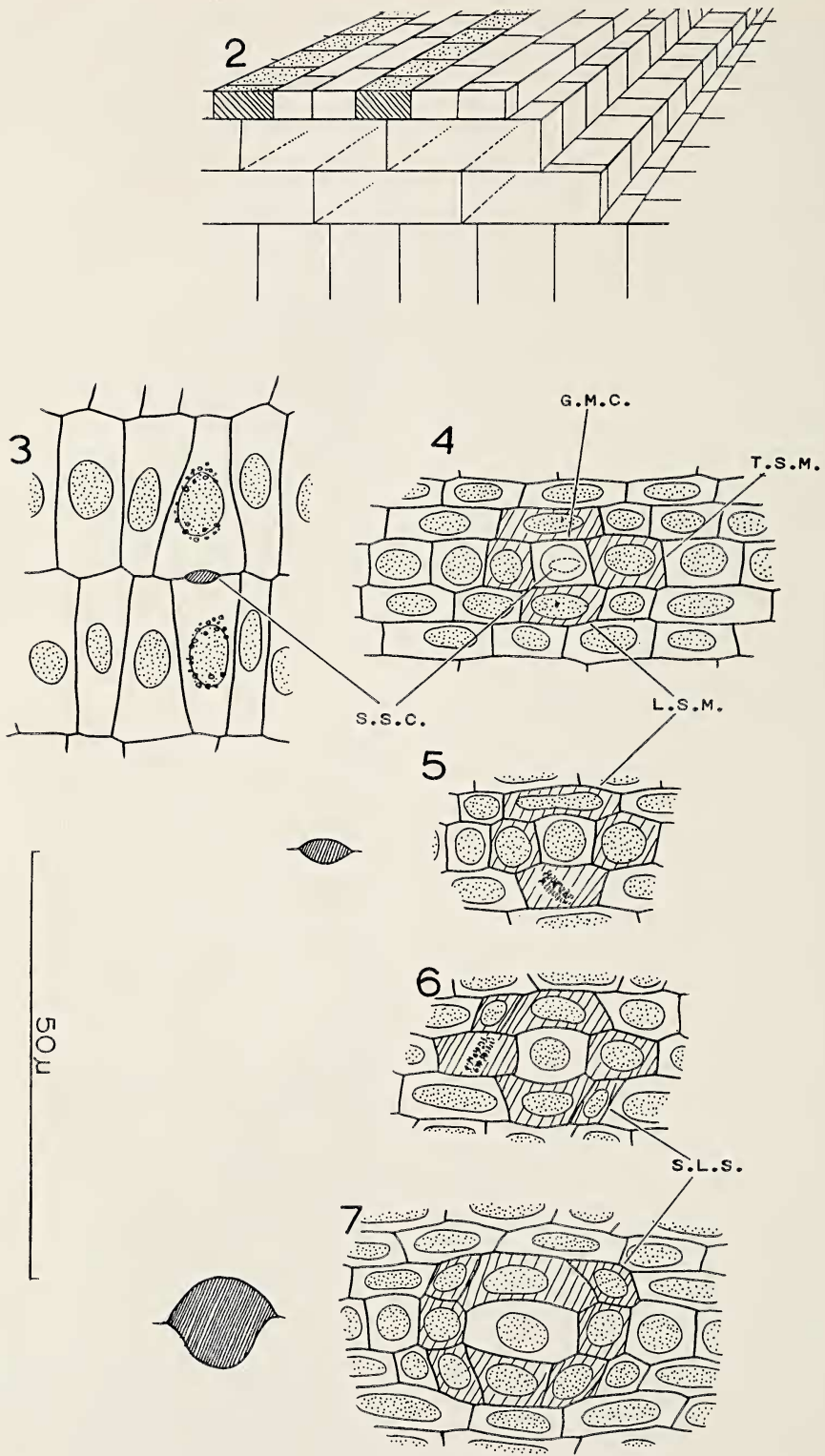
In the subsequent description an attempt has been made to employ a consistent terminology based on that used by Florin (1931). However,

Florin's terminology refers to gymnosperms. Stomata in Pandanaceae are tetracytic in the sense used by Metcalfe (1961). The cells next to the guard cells are subsidiary cells, of which there are four: two lateral subsidiary cells which are very different from normal epidermal cells, and two terminal (or polar) subsidiary cells which are less different from normal epidermal cells. The cells which immediately surround the subsidiary cells may be termed neighbouring cells, but the account of stomatal development below shows that neighbouring cells have different origins. They correspond approximately to the *Kranzzellen* of Florin, who uses the term "neighbouring cell" (*Nachbarzell*) collectively to signify any cell which surrounds the stomatal apparatus and differs from a normal epidermal cell. However, it is difficult to apply Florin's terminology strictly to Pandanaceae. Neighbouring cells in Pandanaceae vary in size and number and may or may not differ in varying degrees from normal epidermal cells.

STRUCTURE OF ABAXIAL STOMATA

Adaxial stomata are often different from abaxial stomata and are described briefly later.

Stomatal development has been described in *Pandanus graminifolius* by Pfitzer (1870) with an emphasis on the position of stomatal mother cells in relation to the hypodermal layers. Pfitzer's observations have been confirmed by my own study of stomatal development in four *Pandanus* species, using the method described by Stebbins and Khush (1961). Stomata always originate from epidermal cell files above the longitudinal anticlinal walls of the outermost hypodermal layer, but directly above the next innermost hypodermal layer (Fig. 2). Transverse divisions continue longest in future stomatal files, so their constituent cells are somewhat shorter, as well as wider, than cells in non-stomatal files. First evidence of stomatal differentiation occurs not in the epidermis itself but in the outermost hypodermal layer, by the development of substomatal chambers below future guard-cell mother cells (guard mother cells). Pfitzer emphasises this peculiarity and suggests that development of this internal cavity determines the presence of a stoma. The two hypodermal cells between which the chamber



originates (Fig. 3) are somewhat larger than adjacent hypodermal cells, have large nuclei, but most conspicuously include a fine granular deposit by which they may be recognized even before the chamber appears. Guard mother cells are recognizable by their position immediately above a chamber, but are not otherwise cytologically different from neighbouring cells of the stomatal file (Fig. 4). They only divide once after becoming recognizable, by the longitudinal wall which produces the guard cells, this usually being the last division in the development of the stomatal complex. Size of substomatal chamber gradually increases as the stomatal complex develops and is a useful indicator of the level of maturity of the complex (cf Figs. 5, 7).

Transverse divisions may continue in those cells of the stomatal file which are not guard mother cells. Such divisions in cells situated at each pole of the guard mother cell produce the terminal subsidiary cells (Fig. 6). These divisions are never synchronous and may occur early or late, but usually are completed before the divisions which delimit lateral subsidiary cells. Cells belonging to files on each side of the guard mother cell produce lateral subsidiary cells. Two successive divisions occur at opposite ends of each cell, each division cutting off by an oblique wall a small cell which is remote from the guard mother cell from a much larger cell next to the guard mother cell (Fig. 5). Rarely divisions in this lateral subsidiary mother cell are longitudinal and not oblique. The last division in the stomatal complex is almost invariably the longitudinal division which produces the guard cells, although rarely divisions producing terminal subsidiary cells do occur later than this.

Development of stomata does not follow a strict acropetal succession, and stomata at different stages of development occur in a small

area of the leaf. In general, however, divisions which produce terminal subsidiary cells are completed first; divisions producing lateral subsidiary cells, which occur throughout a relatively wide region, are completed second; and divisions which produce guard cells are usually last. Divisions within a single complex are rarely synchronous, so that only one division figure per stoma is usually seen.

Division in the guard-cell mother cell is associated with further internal development. As soon as guard cells are produced, but before the stomatal pore opens, enlargement of the substomatal chamber by separation within the second hypodermal layer occurs. When the stomatal pore opens there is thus communication with the internal leaf atmosphere.

This type of stoma corresponds to neither of the two main types recognized by Florin (1931) in gymnosperms, although in most resembles the amphicyclic. It is neither similar to development described by Stebbins and Khush in *Juncus* and *Sagittaria*, nor is it similar to that in *Tradescantia* as these authors suggest.

Because of the constant disposition of stomata in relation to the two outermost hypodermal layers and because of regular partitioning in these layers, the outer hypodermal cells which immediately surround the substomatal chambers have a very uniform shape and arrangement in all Pandanaceae. In the outermost layer the chamber represents a space between longitudinal walls and is enclosed by two U- or V-shaped cells (Fig. 20); in the next innermost layer the chamber represents a space between transverse walls and is surrounded by two kidney-shaped cells at right angles to the cells without (Fig. 21). Further inner hypodermal layers are not uniformly disposed. Variation in stomatal structure largely involves papillae developed on subsidiary and

FIGS. 2-7. Stomatal development in *Pandanus* ($\times 1160$). 2, Diagrammatic representation of surface layers, showing epidermal stomatal files (stippled) in relation to hypodermal layers. 3, Arrangement of cells of outermost hypodermal layer at time of initiation of substomatal chamber. 4, Corresponding arrangement of epidermal cells immediately above Figure 3, stomatal complex recognized largely by position. 5, Oblique division in lateral subsidiary mother cell. 6, Transverse division in terminal subsidiary mother cell at a time when one division has occurred in each lateral subsidiary mother cell. 7, Arrangement of cells in stomatal complex prior to last division, that of the guard mother cell.

In Figures 4-7 the cells surrounding the guard mother cell are cross-hatched for ease of recognition. They are not distinguished in any obvious cytological way. Inset in Figures 5 and 7 shows size of corresponding substomatal chamber: s.s.c., substomatal chamber; g.m.c., guard mother cell; t.s.m., terminal subsidiary mother cell; l.s.m., lateral subsidiary mother cell; s.l.s., sister cell of lateral subsidiary cell.

neighbouring cells. Stomata which have no associated papillae represent the unspecialized state. A variety of types with gradually increasing elaboration of stomata can be recognized, culminating in stomata completely obscured by overarching papillae. In this continuous series a number of arbitrary types have been selected, each successive type representing an advance in specialization over the preceding one. However, a complete series of transitional types can be found connecting the arbitrary classes. *Pandanus* has the most variation. *Sararanga* has unspecialized stomata. *Freycinetia* forms rather a different class and is discussed separately.

(a) *Pandanus* and *Sararanga*

Class 1: Unspecialized stomata (Figs. 8–11, 19, 49–51). Each stoma has two guard cells, each guard cell more or less symmetrical in transverse view, with two equal cutinized ledges, each ledge situated above thickenings of the front wall (Figs. 9, 19). The cell lumen is wide and only slightly dumbbell-shaped in longitudinal view. Guard cells and lateral subsidiary cells are deeper than other epidermal cells, but not sunken. Lateral subsidiary cells are thin-walled and conspicuously different from normal epidermal cells. Terminal subsidiary cells are short but otherwise less well differentiated from normal epidermal cells (Fig. 8). Subsidiary and neighbouring cells are never elaborated, apart from slight overarching of lateral by terminal subsidiary cells. This type of stoma has been recorded in the following species:

Sararanga

S. philippinensis *S. sinuosa*

The stomata in the two known species of *Sararanga* are therefore wholly unspecialized (Figs. 49–51).

Pandanus

P. cominsii *P. pulposus*

P. dubius *P. rockii*
P. odoratissimus var. *P. tectorius*
laevis *P. veitchii*

Solla (1884) records them in:

P. amarillifolius *P. pedunculatus*
P. cuspidatus *P. reflexus*
P. furcatus *P. stenophyllus*
P. javanicus *P. veitchii*

and in six unnamed species.

In subsequent stomatal classes, guard cells are identical in general structure, although they may vary considerably in size (cf Figs. 8, 10); an over-all range from 15–40 μ was observed in the material examined by me, corresponding closely to the range described by Solla.

Class 2: Papillose lateral subsidiary cells (Figs. 12–15). The construction described above is repeated, but with the addition of a row of 4–6 prominent papillae on the outer surface of each lateral subsidiary cell. There is no other stomatal elaboration, except for a tendency for terminal to overarch lateral subsidiary and guard cells to a greater extent than in Class 1 (Fig. 13). Degree of stomatal specialization commonly varies on a single leaf. For example, in material of *P. boninensis* and *P. odoratissimus* both Class 1 and Class 2 stomata were observed on the same leaf, together with intermediate stomata in which papillae occurred on one or another of the two lateral subsidiary cells belonging to a single stoma (Figs. 12, 14). A full row of papillae is always developed, never isolated or reduced numbers of papillae. Class 2 stomata have been observed in:

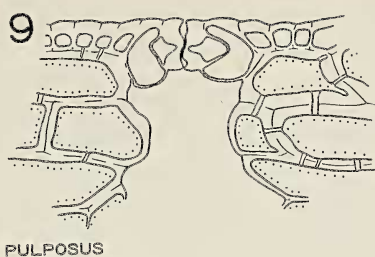
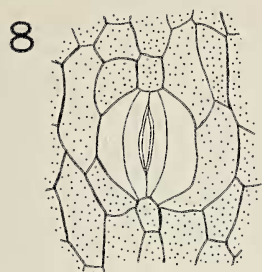
P. baptistii *P. graminifolius* (Pfitzer)
P. boninensis *P. odoratissimus* var.

Solla records them for:

P. furcatus *P. odoratissimus*
P. graminifolius

and in two unnamed species.

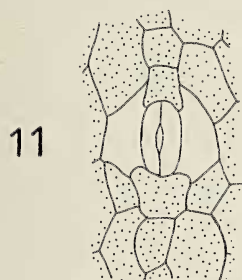
FIGS. 8–21. *Pandanus*. Abaxial stomata classes 1 and 2 ($\times 425$, except 20 and 21, which are $\times 270$). 8, 9, *P. pulposus*. 8, Surface view; 9, transverse section. 10, *P. cominsii*, surface view; guard cells are among the shortest in the genus. 11–13, *P. boninensis*, different types of stomata from same area of single leaf. 14, 15, *P. odoratissimus*, different types of stomata from same area of a single leaf. 16–18, *P. biakensis*. 16, Transverse section; 17, surface view; 18, longitudinal section. 19–21, *P. rockii*. 19, Transverse section; 20, 21, arrangement of hypodermal cells around substomatal chambers; 20, outermost hypodermal layer; 21, next innermost layer.



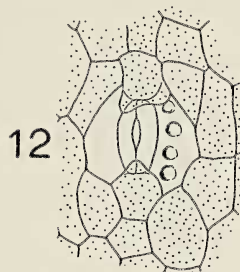
PULPOSUS



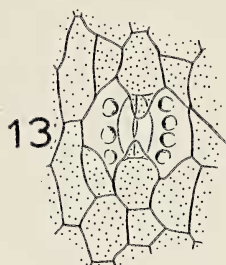
COMINSII



BONINENSIS



12

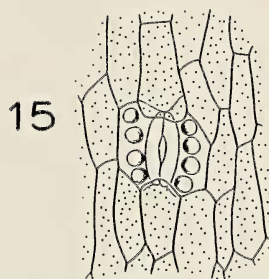


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14

ODORATISSIMUS



15

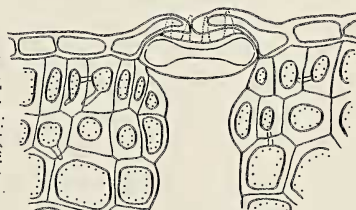


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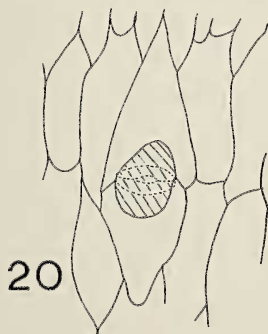
BIAKENSIS



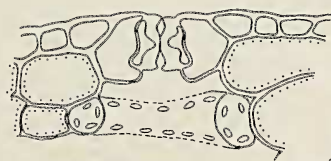
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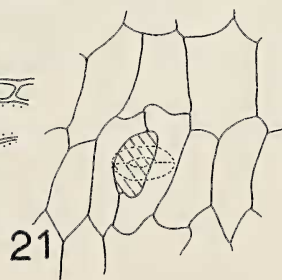
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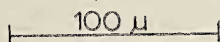
20



19 ROCKII



21



The stomata in *P. boninensis* and *P. graminifolius* are somewhat transitional to the next category.

In all subsequent stomatal classes, papillae on lateral subsidiary cells are developed in exactly the same way, together with other kinds of papillae on other cells. However, the former kind of papillae always remains simple, regardless of elaboration in the latter kind of papillae. Their only variation is in height, taller papillae being up to 25 μ high, as in *P. aurantiacus*.

Class 3: Papillose terminal and lateral subsidiary cells (Figs. 16–18). The slight tendency in Class 2 stomata for the terminal to protrude over the lateral subsidiary cells is much more pronounced in Class 3. This is associated with a tendency for the guard and lateral subsidiary cells to be sunken within the epidermis. Each of the terminal subsidiary cells then has a prominent papilla which overarches the stomatal pore. Frequently papillae from opposite poles meet and overlap, their ends being mutually displaced (Fig. 17), or the papillae may even fork to produce short interdigitating branches. Such papillae are usually adpressed closely to the stomatal pore, between the opposite files of papillae born by lateral subsidiary cells (Fig. 18). Species within this category include:

P. biakensis *P. copelandii* *P. furcatus*

Pandanus biakensis approaches the following type.

Class 4: Papillose neighbouring and subsidiary cells (Figs. 22–33). An advance on Class 3 is represented by the development of papillae which protrude from neighbouring epidermal cells. This augments the outer stomatal cavity produced by sinking of the stomatal apparatus in the epidermis. The relation between papillae associated with stomata and those elsewhere on the epidermis is discussed later. Class 4 stomata are very diverse because the size and frequency of this new kind of papilla varies considerably. In the less elaborate types papillae are not pronounced, as in *P. douglassii* (Figs. 22–24), so that the outer chamber is shallow, but in more

elaborate types the papillae are very tall and form a distinct "stockade" surrounding a very deep outer chamber, as in *P. utilis* (Figs. 28–30). Tall papillae further show a marked tendency to overarch and occlude the outer chamber (e.g., *P. parkinsonii*, Figs. 25–27).

Papillae themselves are diverse. They may form as a result of protrusion of the whole outer wall of the epidermal cell, or involve only part of the outer wall. Less commonly, several papillae may arise from each epidermal cell, as in *P. patina* (Fig. 32). Finally, the more elaborate members of this class show a tendency for the papillae surrounding the stomata to become lobed or shortly branched. Usually this is noticeable first in the terminal subsidiary cells, as in *P. utilis* (Fig. 30), and in this way a transition to Class 5 occurs. In *P. candelabrum* the papillae are very low but often distinctly lobed.

The following species have been included in Class 4, although it is clear from Figures 22–30 that the assemblage is heterogeneous:

<i>P. aimiriikensis</i>	<i>P. parkinsonii</i>
<i>P. candelabrum</i>	<i>P. patina</i>
<i>P. douglassii</i>	<i>P. pistillatus</i>
<i>P. heterocarpus</i>	<i>P. utilis</i>
<i>P. microcarpus</i>	<i>P. whitmeeanus</i>
<i>P. nemoralis</i>	

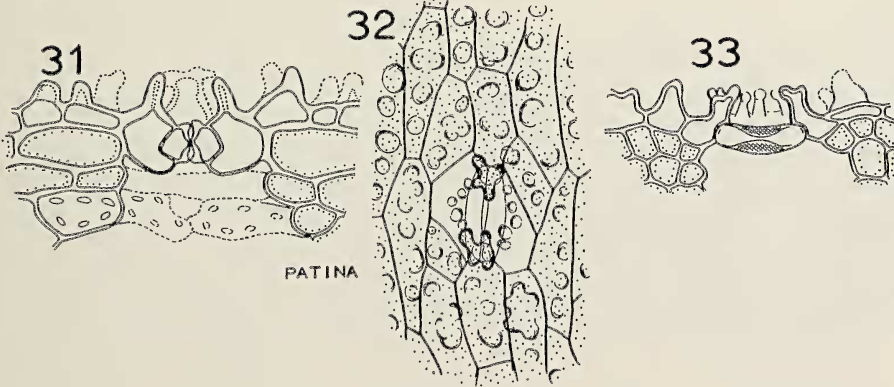
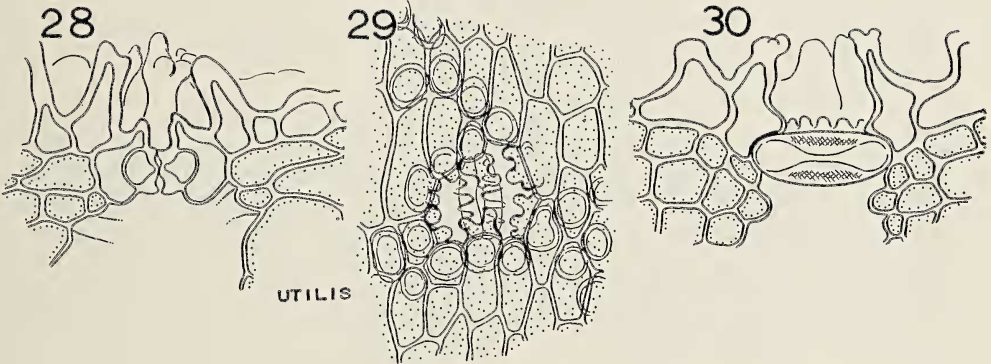
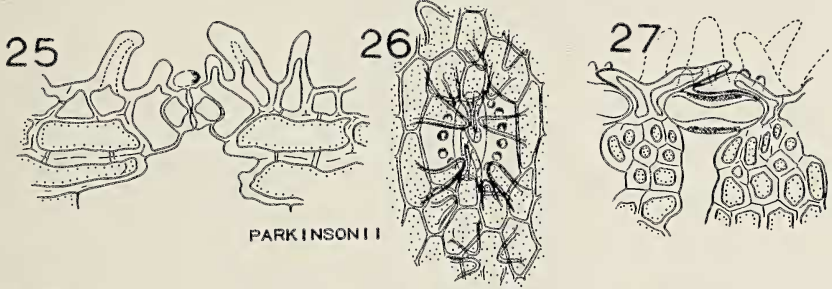
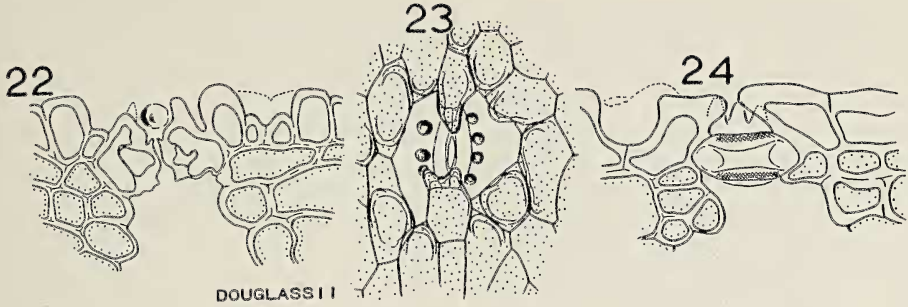
Solla records elaborate stomata in the following species, without making distinction between classes 4 and 5 in the way I have done:

<i>P. caricosus</i>	<i>P. silvestris</i>
<i>P. helicopus</i>	<i>P. tenuifolius</i>
<i>P. microcarpus</i>	<i>P. utilis</i>
<i>P. odoratissimus</i>	

and in 13 unnamed species.

Class 5: Overarching papillae lobed or dendritic (Figs. 34–45). This class includes the most specialized forms. Sinking of the stomata is pronounced, the deep outer stomatal chamber so formed being partly or wholly occluded by the branched papillae of terminal subsidiary and neighbouring cells. In the least elaborate members papillae are short and little lobed, as in *P.*

FIGS. 22–33. *Pandanus* abaxial stomata of classes 3 and 4. 22–24, *P. douglassii*. 22, Transverse section; 23, surface view; 24, longitudinal section. 25–27, *P. parkinsonii*. 25, Transverse section; 26, surface view; 27, longitudinal section. 28–30, *P. utilis*. 28, Transverse section; 29, surface view; 30, longitudinal section. 31–33, *P. patina*. 31, Transverse section; 32, surface view; 33, longitudinal section.



100 μ

capitellatus, which is thus intermediate between classes 4 and 5 (Figs. 34–36). Increasing elaboration can be seen in a number of species whereby the papillae become taller, elaborately lobed distally, the lobes tending to interdigitate and form an incomplete canopy above the outer stomatal chamber (Figs. 40–45). The ultimate condition, and the most elaborate stomatal type in *Pandanus*, was observed in an unidentified species cultivated in Florida in which the canopy was so complete that the guard cells were completely obscured from surface view, a somewhat more elaborate condition than that illustrated in Figure 44.

As in previous classes the papillae arising from terminal subsidiary cells may be either erect or adpressed to the pore (Fig. 42), a feature constant for each species. Where these papillae are adpressed, and indeed to a certain extent in other examples, the outer chamber is partially occluded by papillae which are elevated to three different levels: papillae from the lateral subsidiary cells being shortest, from the terminal subsidiary cells being intermediate, and from the neighbouring cells being tallest (Figs. 40, 43). Clearly, in these most elaborate types water vapour must pass through quite a labyrinth on its way through the epidermis. Species of this category include:

<i>P. archboldianus</i>	<i>P. erinaceus</i>
<i>P. aurantiacus</i>	<i>P. nigridens</i>
<i>P. capitellatus</i>	<i>P. pygmaeus</i>

(b) *Freycinetia*

Freycinetia includes species with unspecialized stomata, corresponding to Class 1 above, and where stomatal elaboration does occur it seems to follow a different trend to that in *Pandanus*, although insufficient material has been examined to be certain of this point. Specialization in *Freycinetia* involves sunken stomata, overarched by simple papillae from terminal and neighbouring cells only. Papillae are never lobed. Rows of papillae on lateral subsidiary cells which are so

characteristic of stomata in classes 2–5 in *Pandanus* have never been observed in *Freycinetia*, except by Solla in one unnamed species (which might be a *Pandanus* misidentified). Further observation is needed to discover if there is a constant difference between *Pandanus* and *Freycinetia* in this respect. Figures 46–48 represent the most elaborate condition seen by me in *Freycinetia*.

The following species, together with six unnamed species listed by Solla, have unspecialized stomata:

<i>F. banksii</i>	<i>F. lucens</i>
<i>F. excelsa</i>	<i>F. mariannensis</i>
<i>F. javanica</i>	

The following has specialized stomata:

F. arborea

Solla records specialized stomata in three unnamed species.

Further peculiarities of epidermal structure in *Freycinetia* are mentioned below.

VARIATION IN STOMATAL STRUCTURE

The above account gives an impression of the range of stomatal structure throughout the Pandanaceae and, at least for *Pandanus*, demonstrates a linear series of increasingly specialized types, each arbitrary class which has been distinguished being more specialized than the preceding. This trend seems to be unidirectional, as can be understood when the stomatal variation in a single individual is considered. A number of ancillary observations need to be outlined first.

(i) *Occluded stomata*. Frequently stomata were observed which stain intensively with safranin, in contrast to normal stomata. These evidently correspond to the "lignified" stomata of Köfler (1918). Such stomata become occluded by resinous or tanniferous material which plugs the stomatal pore. Subsequently the guard cells die and their back walls collapse against the front walls. Usually the substomatal

FIGS. 34–45. *Pandanus* abaxial stomata of classes 4 and 5 (except 39, which shows an adaxial stoma). 34–36, *P. capitellatus*. 34, Transverse section; 35, surface view; 36, longitudinal section. 37, 38, *P. pygmaeus*. 37, Transverse section; 38, surface view. 39–42, *P. archboldianus*. 39, Adaxial stoma, surface view; 40, transverse section; 41, surface view; 42, longitudinal section. 43–45, *P. nigridens*. 43, Transverse section; 44, surface view; 45, longitudinal section.



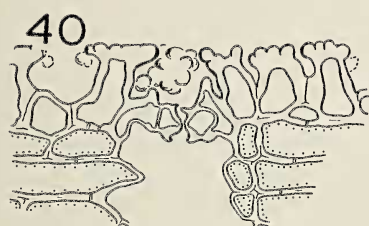
CAPITELLATUS



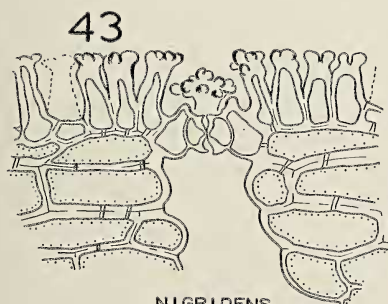
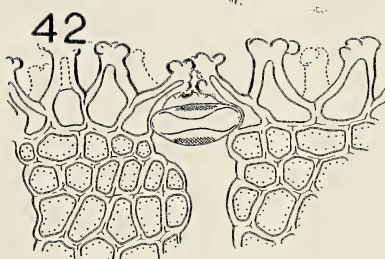
PYGMAEUS



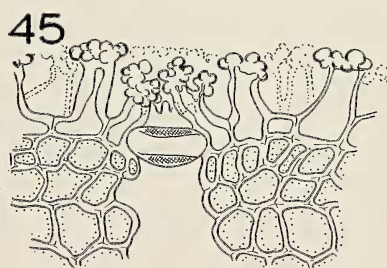
ARCHBOLDIANUS



ARCHBOLDIANUS



NIGRIDENS



100 μ

hypodermal cells put out tylose-like protuberances which fill the substomatal chamber. These invaginations may eventually become thick-walled and pitted. Positive lignin reactions by which Köfler recognized these stomata are apparently a property of the resinous material which initiates these changes, although walls of hypertrophied hypodermal cells may subsequently become lignified. Such occluded stomata are very irregular in their distribution, often being localized. I have observed them in most species examined, although Köfler lists a number of species in which they were not observed. This is probably not significant taxonomically, since the potentiality for their development in old leaves possibly exists in all species. Whether they are pathological or not is not known.

(ii) *Variation within a single leaf.* Discussion of epidermal papillosity has so far dealt only with cells next to stomata. Papillae are sometimes restricted to cells in the vicinity of stomata, as in *P. candelabrum* and *P. whimseanus*, but are more usually distributed, either uniformly or nonuniformly, over the whole abaxial epidermis. Papillae associated with stomata are usually taller than those elsewhere. When they are uniformly distributed papillae occur equally in costal and intercostal regions, although costal are almost invariably lower than intercostal papillae and are equally developed on all cells (e.g., *P. erinaceus* and *P. patina*). Otherwise they are restricted to intercostal regions. A striking and not infrequent condition is for epidermal papillae to be restricted so that they form a distinct reticulum in surface view, with strands of the "network" meeting about the stomata (e.g., *P. capitellatus* and *P. utilis*, Fig. 29). In general, specialisation of epidermal papillae closely parallels specialisation of stomata, although anomalous instances where it does not are described below.

Stomata are very constant in structure throughout a single leaf surface, but adaxial may differ strikingly from abaxial. Adaxial stomata vary in number but are always fewer than abaxial. They are always unspecialized and never appreciably sunken, even in species with very elaborate abaxial stomata. Consequently, in these species the difference between adaxial and abaxial stomata is very striking (cf Figs. 39, 41).

(iii) *Variation throughout a single individ-*

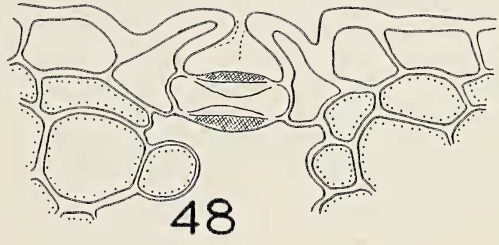
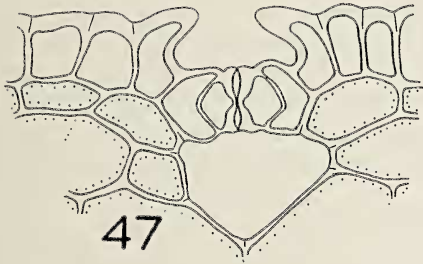
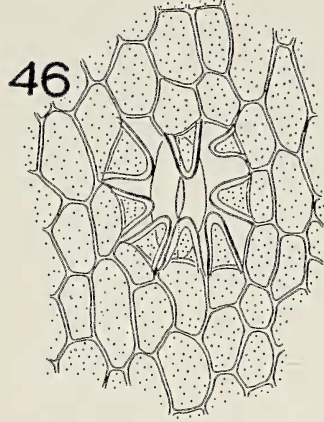
ual. The trend in stomatal elaboration demonstrated throughout *Pandanus* is partly or wholly demonstrated by a single individual in many species. This is because seedling leaves and the first leaves on each renewal branch of the sympodium always possess unspecialized stomata. Thereafter, on subsequent transitional leaves or scale leaves there is a gradual increase in stomatal elaboration culminating in stomata of maximum elaboration developed by a particular species. This maximal condition appears first on the first true foliage (or adult) leaves of a shoot (Fig. 1). Thus on a shoot capable of developing elaborate stomata, a long transitional series of stomatal types can be demonstrated in the scale-leaf series; otherwise, where the species does not develop specialized stomata the range of types on a single shoot may be limited, or there may be only one type. This concept is illustrated diagrammatically in Figure 52. From this it is evident that each species of *Pandanus* has a maximum potential for stomatal elaboration which is achieved gradually on each new shoot. In some species (those which bear Class 5 stomata) the potential is great; in others (those which bear lower class stomata) the potential is limited. Many species have no potential for stomatal elaboration. That this maximum potential is always fully expressed on adult foliage leaves is clear from examination of many samples of a single species from different sources. Thus *P. utilis* always has stomata of the type illustrated in Figures 28–30 on its adult foliage.

Associated with the frequent increase in stomatal complexity on successive leaves of a single shoot is an increase in stomatal number. Prophylls always have very diffusely distributed stomata, in successive scale leaves they become dense, and a more or less constant value is achieved in normal foliage leaves. This is a genuine increase in stomatal index, since epidermal cell size is quite uniform throughout this range of leaf types.

The pattern of stomatal variation described above is familiar in many other plants (e.g., gymnosperms; Florin, 1931). It is easy to demonstrate in *Pandanus* because of the relatively simple linear sequence of increasing stomatal complexity and the constancy of shoot construction which makes leaf categories easy to recognize (Figs. 1, 52).

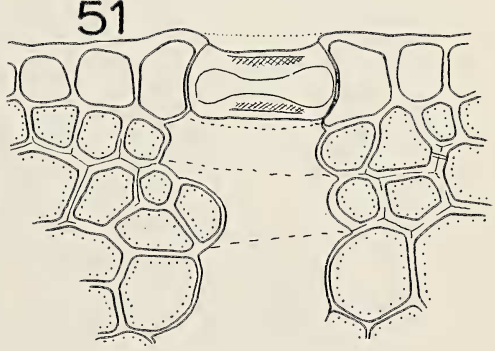
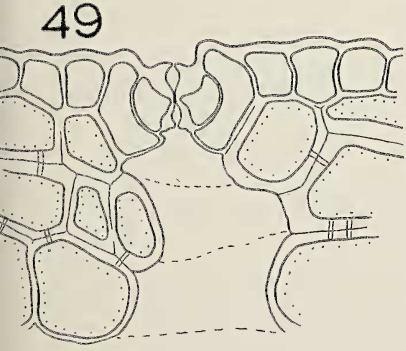
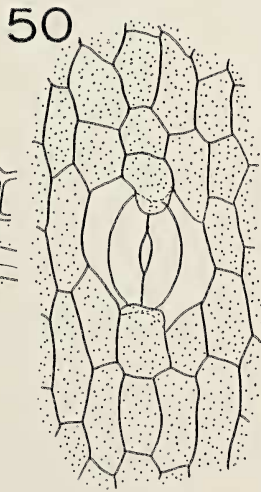
The patterns of variability in stomata have

FREYCISETIA
ARBOREA



100 μ

SARARANGA
SINUOSA



FIGS. 46-51. *Freycinetia* and *Sararanga*, abaxial stomata ($\times 425$). 46-48, *Freycinetia arborea*. 46, Surface view; 47, transverse section; 48, longitudinal section. 49-51, *Sararanga sinuosa*. 49, Transverse section; 50, surface view; 51, longitudinal section.

been described in such a way as to suggest that development of papillae on epidermal cells remote from stomata closely parallels that on cells immediately associated with stomata. This is undoubtedly true for most *Pandanus* species, but there is evidence in *Freycinetia excelsa* and in an unnamed species of *Pandanus* cultivated in Florida that epidermal papillae may proliferate quite independently of stomata. In the former example some shoots had leaves with a distinctly papillose abaxial epidermis, although the stomata remained quite unspecialized. Clearly, there may be independence between epidermal and stomatal papillae in some Pandanaceae, and my account with the emphasis on a linear correlation between the two does not tell the whole story.

DISCUSSION

Patterns of anatomical features in the leaf which are of diagnostic value have not yet

been demonstrated in the Pandanaceae. Stomatal structure, which shows most variation, must be used cautiously for systematic purposes in view of its variation in a single individual. So far there is little indication that stomatal type is taxonomically significant.

Stomata in Pandanaceae vary in a way which might suggest a relation with environmental aridity. It is clear, however, that the degree of stomatal elaboration is under genetic control and is not wholly a consequence of external environment. Evidence is provided by a number of species of *Pandanus* cultivated side by side under identical circumstances in Florida. In this collection the whole range of stomatal types in Classes 1-5 has been seen, so that in this collection the release of stomatal potential is not affected by environment. Conversely, material of *P. dubius* from contrasted environments, supplied by Dr. B. C. Stone, showed no variation in stomatal elaboration.

If it is probable that stomatal elaboration is

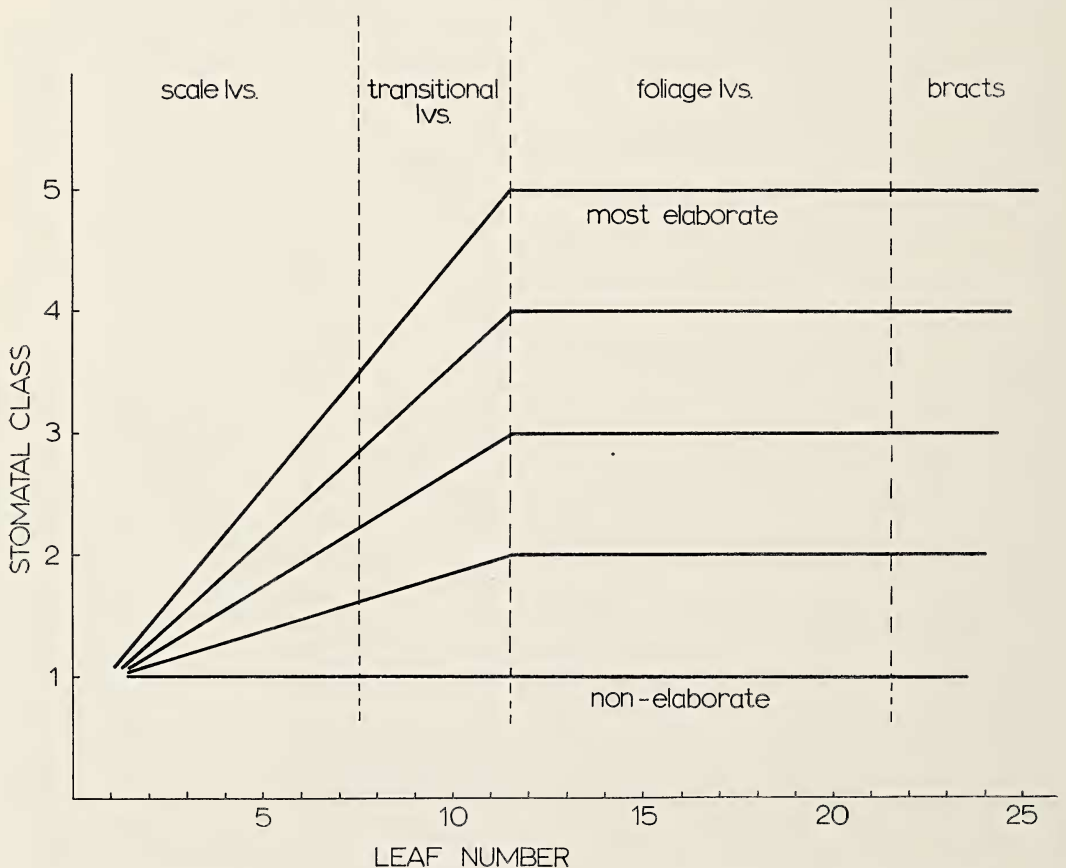


FIG. 52. Diagram representing increasing elaboration of stomata on a single shoot, the ultimate potential differing in different species (cf Fig. 1).

differently expressed in closely related species, it is possible that elaboration is an ecological adaptation which has become fixed genetically. It is an attractive hypothesis to regard elaborate epidermal structures associated in varying degrees with the guard cells as devices whereby transpiration is reduced, since this type of modification is frequent in plants of dry regions. However, in *Pandanus* an increase in epidermal papillosity does not involve a comparable increase in cuticular thickness. Therefore elaborate papillae actually increase surface area and presumably also cuticular transpiration. There may be overcompensation for this by reduction in stomatal transpiration through obstruction of the outer stomatal chamber. A more obvious antitranspiration device is the plugging of many stomata (the "lignified" stomata of Köfler) by resinous deposits, although this mechanism seems irregular.

Stomatal elaboration might therefore be interpreted more readily in terms of ecology and physiology than of taxonomy. *Pandanus* occupies a wide range of habitats (van Steenis, 1956) and it would be valuable to examine stomatal structure of species occupying contrasted habitats. Also the basic physiology of water conduction in *Pandanus* is itself relevant. *Pandanus* has vessel elements with scalariform perforation plates bearing many thickening bars in all parts, elements of the root being somewhat more specialized than those of the leaf and stem. Even if the stem is an efficient water conductor, it is likely to be subject to water stress as it grows older. The leafy crown tends to increase in size by branching, but the main axis, being devoid of a secondary vascular cambium (Schoute, 1907), has no means of increasing the volume of water conducting tissue (Tomlinson, 1964). Reduction of water lost through the leaf surface would evidently be favourable, regardless of the environment.

No one single factor, therefore, can explain stomatal elaboration in Pandanaceae, since it may be correlated partly or wholly with several unrelated factors, notably phylogeny, ecology, and physiology, together with over-all stature and growth form. The limited scope of the present study should now be quite clear, as well as the enormous scope for future observation.

MATERIAL EXAMINED

Pandanus

(i) Dried specimens from Bishop Museum, Honolulu

- P. aimiriikensis* Martelli....St. John 25884
P. archboldianus Merr. and
 PerryBarrett 10203
P. biakensis St. John.....St. John 26142
P. boninensis Warb.....Fosberg 31483
P. capitellatus Merr. and
 PerryStone 2466
P. cominsii Hemsl.....Stone 2549
P. douglassii Gaud.....Pearsall 74
P. dubius Spreng.....St. John 25898
P. ernaceus B. C. Stone.....Stone 2578
P. nemoralis Merr. and Perry..Stone 2483
P. nigridens B. C. Stone.....Stone 2539
P. odoratissimus L.f. var.....Doty 16791
P. parkinsonii Martelli.....Stone 2615
P. pistillatus Martelli.....Stone 2576
P. pulposus (Warb.)

MartelliStone 1110

P. whitmeeanus Martelli.....Stone 2209

(ii) Other sources

Cultivated in Florida:

P. baptistii Hort.

P. copelandii Hort.

P. utilis Bory

Cultivated at Foster Botanic Garden, Honolulu, Hawaii:

P. odoratissimus L.f. var. *laevis*

P. patina Martelli

P. c.f. pygmaeus Thou.

P. rockii Martelli

P. sp. ("Hala")

Slide collection, Jodrell Laboratory, Royal Botanic Gardens, Kew:

P. aurantiacus Ridl.

P. furcatus Roxb.

P. heterocarpus Balf.

P. microcarpus Balf.

P. tectorius Sol.

P. utilis Bory

Slide collection, Professor V. I. Cheadle, at University of California, Davis, California:

P. utilis Bory.....Atkins Garden,
 Cienfuegos, Cuba

P. veitchii Hort.....Atkins Garden,
 Cienfuegos, Cuba

Collections from wild:

P. candelabrum Beauv.....Akofokyir,
Cape Coast, Ghana

Freycinetia

Collections from wild:

F. arborea Gaud.....Poamoho Trail,
Oahu, Hawaii

F. mariannensis Mert...Stone 4217, Guam
Slide collection, Jodrell Laboratory, Royal
Botanic Gardens, Kew:

F. banksii Cunn.

F. javanica Bl.

F. lucens Ridl.

Slide collection, Professor V. I. Cheadle, at
University of California, Davis, California:

F. excelsa F. Muell.....CA. 216 and 230
Atherton and Cairns, Australia

Sararanga

Dried specimen from Herbarium, Royal
Botanic Gardens, Kew:

S. philippinensis Merr.....Ramos and
Edaño 48047, Philippines

Collection from wild:

S. sinuosa Hemsl.....G. F. C. Dennis 340,
British Solomon Islands

ACKNOWLEDGMENTS

Technical assistants employed during this study were financed by a grant from the American Philosophical Society. I wish to thank Mr. J. B. Hall for material of *Pandanus candelabrum*, Mr. G. C. Dennis for material of *Sararanga sinuosa*,² Mr. L. L. Forman for material of *Sararanga philippinensis*, Dr. C. R. Metcalfe and Dr. V. I. Cheadle for access to slide collections in their respective charge, Dr. Murray Gaskin for permitting unlimited access to plants cultivated at the U. S. Dept. of Agriculture Experiment Station, Old Cutler Road, Miami, and Professor T. M. Harris, F.R.S., for several helpful suggestions. I owe a particular debt to Dr. B. C. Stone, College of Agaña, Guam, who originally made this study feasible and for his subsequent interest.

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² It is hoped to describe in detail the anatomy of *Sararanga* in a future account based on Mr. Dennis's collections.

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